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85

(54) Title: CEREBROVASCULAR AMYLOID PROTEIN-SPECIFIC MONOCLONAL ANTIBODY SV17-6E10

(57) Abstract

Disclosed is monoclonal antibody SV17-6E10 and a specific-binding fragment thereof which is specifically reactive with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin. Also disclosed is a hybridoma cell line capable of producing the monoclonal antibody, a reagent composition which incorporates the monoclonal antibody or specific-binding fragments thereof and an immunoassay method for their use.

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Cerebrovascular amyloid protein-specific monoclonal antibody 3V17-6E10

BACKGROUND OF THE INVENTION

Field Of The Invention

The present invention relates to neurological disorders, and, more particularly, to antibodies that are specific for peptides associated therewith.

Brief Description Of The Prior Art

Alzheimer's disease is characterized by three major pathological markers. They are neuritic plaques, neurofibrillary tangles and cerebrovascular amyloidosis. Glenner, et al., Biochem. Biophys. Res. Comm., 120:885 (1984) and Wisniewski, et al., Int. Symp. Dementia and Amyloid "Neuropathology", Suppl. 3:87 (1986). The accumulation of two types of aberrant fibrils, the paired helical filaments and the amyloid are characterized neuropathologically in Alzheimer's disease/senile dementia of the Alzheimer type. Plaque and cerebrovascular amyloid are primarily made up of a 40- to 42- amino acid residue peptide, called the B-peptide.

Alzheimer's cerebrovascular amyloid protein has been purified and a 24 amino acid residue sequence has been reported. Glenner, et al., Biochem. Biophys. Res. Comm., 122:1131 (1984). It has also been observed that the sequenced portion of the cerebrovascular amyloid peptide is part of the sequence of a precursor protein. Robakis, et al., Proc. Nat. Acad. Sci. USA, 84:4190 (1987) and Robakis, et al., The Lancet, 1:384 (1987).

Down's Syndrome is a disability characterized by the inheritance of an extra copy of chromosome 21 in each cell. Older persons afflicted with Down's

Syndrome display dementia that resembles Alzheimer's disease. The cerebral tissues of these individuals exhibit the same neuropathological findings of Alzheimer's disease, i.e., amyloid-containing neuritic plaques, neurofibrillary tangles and cerebrovascular amyloidosis. The amyloid deposits of Down's Syndrome contain the same peptide as the amyloid deposits found in the brains of Alzheimer's disease victims. See, Glenner et al., Biochem. Biophys. Res. Comm., 122:1131 (1984).

As applied to other peptides and antigens, including many associated with various diseases, monoclonal antibody products of hybridoma technology has often proven valuable in studying factors associated with these diseases and in diagnosis of the disease in clinical settings. Kim, et al., Neuroscience Research Communications, 2:121 (1988) discloses production and characterization of monoclonal antibodies specific for the synthetic cerebrovascular amyloid protein described in Glenner et al., Biochem. Biophys. Res. Comm., 122:1131 (1984), supra.

SUMMARY OF THE INVENTION

Hybridoma technology is one of the most important biological tools for the analysis of complex antigens. Using the SV17-6E10 monoclonal antibodies of the present invention, which are specific to a recently acquired amino acid sequence of the beta-amyloid precursor protein, it has become possible to study the beta-amyloid precursor protein which leads to significant deposits of vascular and neuritic amyloid plaques in Alzheimer's disease and Down's syndrome. These monoclonal antibodies provide the potential for non-invasive diagnosis of Alzheimer's

disease using body fluids such as spinal fluid, serum or urine.

In one aspect, the invention provides a monoclonal antibody, designated SV17-6E10, and antigen - binding fragments thereof which are specifically reactive with a peptide whose concentration level is elevated in individuals having Alzheimer's disease or Down's syndrome as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin. The monoclonal antibodies of the invention are of subclass IgG_1. Papain-digestion produced antigen-binding fragments (e.g. Fab) have also been shown to specifically recognize amyloid plaque.

The monoclonal antibody is produced by a hybridoma formed by fusion of cells from a myeloma line, usually of mouse origin, and antibody-producing cells, also usually of mouse origin, previously immunized with a peptide whose concentration level is elevated in individuals having Alzheimer's disease or Down's syndrome as compared to individuals of substantially the same age who are not so-afflicted and which do not react with other peptides of human origin, such as one which forms a portion of a cerebrovascular amyloid protein characteristic of Alzheimer's disease or Down's syndrome. Preferably, a mouse NSO myeloma line is used, particularly with antibody-producing cells from a mouse previously immunized with a peptide comprising the sequence comprising asp - ala - glu - phe - arg - his - asp - ser - gly - tyr - glu - val - his - his - gln - lys - leu. Further in this regard, the invention provides a hybridoma cell line capable of producing the monoclonal antibodies described above.

In another aspect, the invention provides a composition for quantitatively determining a peptide whose concentration level characteristic of Alzheimer's disease or Down's syndrome in an individual. The composition comprises a monoclonal antibody or antigen - binding fragment thereof in accordance with the invention and a detectable moiety which is directly or indirectly associated therewith. In one embodiment, the monoclonal antibody is conjugated to an enzyme and the detectable moiety comprises a chromogenic redox substrate for the enzyme. In another embodiment, the monoclonal antibody is conjugated to one partner of a specific binding pair and the other partner of the specific binding pair is conjugated to a detectable moiety or a substance capable of rendering the moiety detectable. One example of this embodiment is where the monoclonal antibody or antigen - binding fragment thereof additionally serves as an antigen of the specific binding pair. The partner for the monoclonal or fragment is an anti-immunoglobulin antibody, usually anti - IgG and usually from a species other than that of its partner, which is labeled, such as with a fluorophore like fluorescein isothiocyanate. Another example is where one partner of the specific binding pair is selected from biotin and its binding analogs and the other partner is selected from avidin and its binding analogs. Usually, the detectable moiety is a chromophore, fluorophore or luminophore and the substance capable of rendering it detectable is an energy donor or catalyst therefor.

Further in this regard, the invention provides an immunoassay method. The method comprises contacting a sample, from the individual suspected of having Alzheimer's disease or Down's syndrome, with

the composition of the invention and quantitatively observing any detectable response.

Monoclonal antibody SV17-6E10 is very unique in that no monoclonal antibody reactive to this peptide (17 amino acid residue) has previously been reported. Monoclonal antibody SV17-6E10 has been used, as exemplified below, in immunoassays for characterizing the amyloid peptide and precursor amyloid peptides in connection with biogenesis of B-amyloid peptide plaques found in Alzheimer's disease and Down's syndrome afflicted brains.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1(a) shows amyloid plaques stained with SV17-6E10 monoclonal antibody.

Fig. 1(b) shows a serial section stained with SCVAP-2F9 monoclonal antibody. A serial section stained with SCVAP-4G8 monoclonal antibody produced results identical to those shown in Fig. 1(b).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Monoclonal Antibody SV17-6E10 to Amyloid Peptide

In the experiments reported by this example, a synthetic peptide, an antigenic conjugate thereof and tryptic fragments of the peptide were used to prepare and characterize the monoclonal antibody of the present invention.

17 Amino Acid Residue Synthetic B-Amyloid Peptide (SV17)

Preparation of SV17 was as follows. A 17-residue synthetic cerebrovascular amyloid peptide (SV17), having the sequence asp - ala - glu - phe - arg - his - asp - ser - gly - tyr - gln - val - his -

his - glu - lys - leu, corresponding to a portion of the published sequence of the Alzheimer's cerebrovascular amyloid peptide was obtained from Biosearch Laboratories, San Rafael, CA. The sequence mentioned was published in Glenner, et al., Biochem. Biophys. Res. Comm., 120:885 (1984). The preparation as received was purified by reverse phase liquid chromatography. The purified SV17 peptide (1 mg) was conjugated to keyhole-limpet hemocyanin (6 mg) with glutaraldehyde (3 ul) in sodium phosphate (1.2 ml, 50 mM, pH 7.5) at 4°C for 2 hours to provide the SV17-KLH antigen.

Preparation of Hybrid Cell Lines

Procedures for producing antibody-secreting hybrid cell lines as described in Togashi, et al., Arch. Virol., 67:149 (1981) and modified as described in Kim, et al., J. Clin. Microbiol., 18:331 (1983) were applied as follows.

BALB/CJ female mice (Jackson Laboratories, Bar Harbor, ME) were immunized via the back foot pads with Ribi adjuvant (200 ul) (Ribi Immunochem Research, Inc., Hamilton, MT) containing SV17-KLH antigen (25-50 ug). At 21 days after the initial injection, these mice were immunized intraperitoneally with SV17-KLH antigen (25-50 ug) in Ribi adjuvant (200 ul) four times at about four week intervals. Four days prior to fusion (at six weeks after the last immunization) the mice received intraperitoneal booster injections of SV17 (200 ug) without adjuvant. Immune spleen cells were harvested as described in Galfre, et al., Methods Enzymol., 3:73 (1981). A mouse with the highest titer after SV17-KLH antigen immunization was selected for hybrid clone production.

NSO myeloma cells were obtained from Dr. Choi, Kyung Hee of Albert Einstein Medical School, New York, NY. The immune spleen cells were incubated with the NSO myeloma cells and fused at a ratio of 5:1 in the presence of polyethyleneglycol 1500 (Boehringer Mannheim, Indianapolis, IN) as the fusing agent. The cells were maintained in Iscoves modified Dulbecco minimum essential medium containing 15% fetal bovine serum, 10^{-4} M hypoxanthine, 1.6 x 10^{-5} M thymidine and 4.0 x 10^{-7} M aminopterin for 14 days. Subsequent feedings with medium containing only hypoxanthine and thymidine were performed on days 18, 22 and 25. After day 25, cells were fed with medium that did not include hypoxanthine, thymidine or aminopterin. Normal BALB/CJ mouse macrophages (6,000 cells per well in 96 wells of a microtiter plate) as a feeder layer were used for the initial hybridoma production and also for subcloning the selected hybrids by limiting dilutions.

Establishment of Hybrid Clones Secreting Anti-SV17 mABs

After the initial hybridoma production, as described above, 5 x 10^4 hybridoma cells in 1.0 ml were added to each well of 96 well tissue culture plates. Hybrid cell growth was detected by the ELISA technique using the synthetic peptide antigen (1 ug/ml) coated on the wells. Supernatant fluid in several wells were found to contain SV17 - specific antibody.

The hybrid that produced SV17-6E10 antibody, as determined by the ELISA procedure, was established and cloned three times by limiting dilutions to obtain a pure clone. Normal BALB/CJ mouse macrophages (6,000 cells per well in 96 wells of a microtiter plate) as a feeder layer were used here

also. Stable cloned hybrid cell lines were established and are referred to here as purified antibodies.

Example 2

Monoclonal Antibody SV17-6E10

Immunocytochemistry Reagent

Monoclonal antibody SV17-6E10 was used in the experiments reported by this example as an immunocytochemistry reagent. Paraffin -embedded, formalin - fixed Alzheimer and normal control cortex sections (6 um) were stained using the avidin-biotin complex technique (Bethesda Research Laboratories, Bethesda, MD). Amyloid present in neuritic plaques and cortical and meningeal vessels in brain sections of Alzheimer's patients were stained and those of non-afflicted controls were not. No staining of Alzheimer neurofibrillary tangles or axons/neurofilaments either in tissue sections or in isolated neurons were observed with monoclonal antibody SV17-6E10.

The immunoreactivity of monoclonal antibody SV17-6E10 was then compared with that of SVCAP-2F9 (or SVCAP-4G8) on the amyloid plaques. Comparison of their immunoreactivities with amyloid plaques using serial sections (6 um thickness) revealed that there were two types of amyloid plaques present. Regardless of high concentration of SV17-6E10 used, one group of plaques was lightly stained while the other group of plaques was darkly stained (Fig. 1a). When SCVAP-2F9 or SCVAP-4G8 monoclonal antibody was used, these two groups of plaques all reacted with equal staining intensity (Fig. 1b). Monoclonal antibody SV17-6E10 was raised against the amino acid residue 1-17 of the synthetic amyloid B-protein while SCVAP-2F9 and SCVAP-4G8 are monoclonal antibodies

specific to an epitope in the 17-24 amino acid segment of the synthetic amyloid B-protein. Judging from these observations, the amount of the peptide with amino acids 1-17 was not equally present in these plaques. However, the amount of B amyloid protein having amino acids 17-24 was approximately the same in all the plaques as evidenced by equal staining density. The availability of SV17-6E10 and SCVAP-2F9 or SCVAP-4G8 makes it possible to reveal for the first time that the plaques are composed of more peptides with amino acid residues 17-24 and less of the peptide having amino acid residues 1-17 in some plaques.

The immunohistochemical staining procedure used was as follows. The deparaffinized sections (6 um thickness) were washed in 0.05M Tris-based buffer (TBS) (pH 7.6) and 0.15M NaCl (TBS) for 5-10 minutes and immersed in undiluted 99% formic acid (Sigma Chemical Co., St. Louis, MO) for 3-4 hours. They were then washed with tap water (3-4 times) and then washed with TBS (5-6 times). They were further immersed in 0.3% hydrogen peroxide for 20 minutes and washed with TBS (5-6 times). After treatment with normal horse serum (Vector stain Elite ABC Kit, anti-mouse IgG made in horse) for 30 minutes, sections were incubated with primary mouse monoclonal antibody (SCVAP-2F9, SCVAP-4G8 or SV17-6E10) to amyloid B-protein (diluted 1:50-4,000) overnight in a moist chamber at room temperature. All slides were washed with 0.05 M TBS after each incubation. Secondary antibody (biotinylated anti-mouse IgG, Vector Laboratories, Inc., Burlingame, CA) was applied for 30 minutes. Avidin-biotinylated peroxidase (Vector-Stain Elite ABC Kit) was used for 45 minutes. They were then stained with 3,3' -diaminobenzidine tetrahydrochloride (DAB) for 5-10

minutes. Sections were lightly stained with Hematoxylin (RICCA Chemical Co., Arlington, TX) for 30 seconds, dehydrated and covered with permount and cover slips.

Example 3

Fab Fragments Of Monoclonal Antibody SV17-6E10

Fab fragments of monoclonal antibody SV17-6E10 were prepared and tested as follows. Immunoglobulin IgG purified by protein A column was concentrated to 20 mg/ml in 20 mM phosphate/10 mM EDTA. A portion (0.5 ml) was mixed with digestion buffer (0.5 ml; 42 mg cysteine - HCl in 12 ml phosphate buffer; pH 7.0). Then, a portion (0.5 ml) of 50% slurry of immobilized papain, which had been rinsed twice with digestion buffer, was incubated (5 hours at 37°C) with constant shaking. The solubilized Fab and Fc fragments and undigested IgG from the immobilized papain gel were recovered. The recovered supernatant was applied to a protein A column which had been equilibrated with binding buffer. Eluate contained Fab fragments.

The Fab fragments were dialyzed against PBS and used for immunostaining of brain sections. When avidin/biotin complex staining method or FITC immunofluorescent staining procedures were used, the Fab fragments of SV17-6E10 selectively stained only amyloid plaques.

Example 4

Double Antibody Sandwich Immunoassay

In the experiments reported by this example, B-amyloid protein was detected using the double antibody sandwich procedure described in Kim, et al., J. Clin. Microbiol., 18:331 (1983).

Falcon ELISA plates with 96 wells were coated with purified SV17-6E10 monoclonal antibody (50 ul; 5 ug/ml in carbonate buffer; pH 9.6) overnight at 4°C. After the plates were washed 10 times with distilled water, they were coated with PBS containing 10% goat serum, 3% bovine serum albumin, and 1% Triton X-100 (PBSTGB) for one hour at room temperature; washed 10x again with distilled water; were then coated with 50 ul of the 24 amino acid residue synthetic B-protein (referred to as "SCVAP2"; sequence - DAEFRHDSGYEVHHQKLFAEDV); diluted in PBSTGB; and were thereafter incubated for 2 hours at room temperature. Note that amino acid residue 11 is glutamine here.

After the plates were washed, the optimum dilution of horseradish peroxidase conjugated monoclonal antibody 4G8 (4G8-HRP) at 1/1,600 dilution was added and plates were incubated an additional 2 hours at room temperature. The plates were then washed for 20 minutes with PBST and a final wash with distilled water, and orthophenylene diamine (OPD) substrate solution (100 ul) was added. After 10 minutes, 75 ul of 1 M H_2SO_4 was added and the color change was determined with a Dynatech Microelisa Auto Reader MC640 with a 490 nm filter. A well that received only PBSTGB was used as a negative control. The cutoff point between positive and negative was the average optical density value of the negative controls plus 2 standard deviations. As reported below, SV17-6E10:4G8HRP refers to the reagent wherein SV17-6E10 is used as the capture antibody and 4G8HRP is the detection antibody. Alternatively, in the other embodiment reported, SCVAP-4G8 is the capture antibody and SV17-6E10HRP is the detecting antibody. The results are reported in Table 1.

Table 1

Double Antibody ELISA Test

	SCVAP2 (picog/ml)	6E10:4G8HRP Relative OD Reading	4G8:6E10HRP Relative OD Reading
1.	500,000	658	1533
2.	250,000	662	1365
3.	125,000	786	1284
4.	62,500	694	1268
5.	31,250	756	1275
6.	15,625	610	681
7.	7,812.50	400	553
8.	3,906.25	222	274
9.	1,953.12	174	173
10.	976.56	125	82
11.	488.28	94	40
12.	244.14	9	23
13.	122.07	21	6
14.	61.04	28	9
15.	30.52	19	1
16.	15.25	31	7
17.	7.63	49	27
18.	3.81	43	35
19.	1.91	52	8
20.	0.95	51	0

6E10:4G8HRP ELISA Sensitivity	4G8:6E10HRP ELISA Sensitivity
Mean = 33.7	Mean = 11
Std. Dev. = 15.7	Std. Dev. = 13
Mean + Two Std. Dev. = 65.1	Mean + Two Std. Dev. = 37.0

Positive results were defined as the highest dilution of B-protein giving an optical density value higher than the cut-off optical density value. Both assay systems are capable of detecting as little as 488.28 picograms of B-amyloid synthetic peptide per ml or 24.4 picogram of B-amyloid synthetic peptide present in 50 ul of sample. Therefore, as long as 6E10 and 4G8 were used in combination of either detecting or capture antibody the double antibody sandwich ELISA was a very sensitive test. This successful double antibody sandwich ELISA system for detecting B-protein has never been reported before.

What Is Claimed Is:

1. Monoclonal antibody SV17-6E10 and antigen - binding fragments thereof.

2. The monoclonal antibody or binding fragment of claim 1 which is specifically reactive with a cerebrovascular amyloid protein whose elevated levels are characteristic of Alzheimer's disease.

3. The monoclonal antibody or binding fragment of claim 1 which is of subclass IgG1.

4. The monoclonal antibody or binding fragment of claim 1 which is specifically bindable with a peptide comprising the sequence asp - ala - glu - phe - arg - his - asp - ser - gly - tyr - gln - val - his - his - gln - lys - leu.

5. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin.

6. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and antibody-producing cells from a mouse previously immunized with a cerebrovascular amyloid protein whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome.

7. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted, and which monoclonal antibody or binding fragment thereof does not react with other peptides of human origin.

8. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a cerebrovascular amyloid protein whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome.

9. A monoclonal antibody which is produced by a hybridoma formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide comprising the sequence asp - ala - glu - phe - arg - his - asp - ser - gly - tyr - gln - val - his - his - gln - lys - leu or an antigen - binding fragment of said monoclonal antibody.

10. Monoclonal antibody SV17-6E10 or an antigen - binding fragment of said monoclonal antibody derived from hybridoma cells formed from NSO myeloma cells and spleen cells of a Balb/CJ mouse immunized with a peptide having the sequence asp - ala - glu - phe - arg - his - asp - ser - gly - tyr - gln - val - his - his - gln - lys - leu which is conjugated directly or

through a linkage group to keyhole - limpet hemocyanin.

11. A hybridoma cell line capable of producing the monoclonal antibody of claim 1.

12. The hybridoma cell line of claim 11 which is formed by fusion of cells from a myeloma line and antibody-producing cells previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

13. The hybridoma cell line of claim 11 which is formed by fusion of cells from a mouse myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

14. The hybridoma cell line of claim 11 which is formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted.

15. The hybridoma cell line of claim 11 which is formed by fusion of cells from a mouse NSO myeloma line and antibody-producing cells from a mouse previously immunized with a peptide comprising the sequence asp - ala - glu - phe - arg - his - asp -

ser - gly - tyr - gln - val - his - his - gln - lys - leu.

16. A composition for quantitatively determining a peptide whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome in an individual, which composition comprises the monoclonal antibody or specific - binding fragment of claim 1 and a detectable moiety which is directly or indirectly associated therewith.

17. The composition of claim 16 wherein said monoclonal antibody or specific - binding fragment is conjugated to an enzyme and said detectable moiety comprises a chromogenic redox substrate for said enzyme.

18. The composition of claim 16 wherein said monoclonal antibody or specific - binding fragment is specifically bindable with a substance attached to said detectable moiety.

19. The composition of claim 18 wherein the substance attached to said detectable moiety is an antibody specifically bindable with an immunoglobulin.

20. The composition of claim 16 wherein said monoclonal antibody is conjugated to one partner of a specific binding pair and which further comprises the other partner of said specific binding pair conjugated to said detectable moiety or a substance capable of rendering said moiety detectable.

21. The composition of claim 20 wherein one partner of said specific binding pair is selected from the group of biotin and its binding analogs and the other

partner of said binding pair is selected from the group of avidin and its binding analogs.

22. The composition of either of claims 20 or 21 wherein said detectable moiety is a chromophore, fluorophore or luminophore and said substance capable of rendering said moiety detectable is an energy donor or catalyst therefor.

23. A method for quantitatively determining a peptide whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome in an individual, which method comprises contacting a sample from said individual with the composition of any of claims 16-20 and quantitatively observing any detectable response.

FIG. 1 (a)

FIG. 1 (b)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02003

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12N 5/20; C07K 15/28; G01N 33/533,534,535

U.S. CL: 530/387, 388; 435/240.27, 7, 188

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
U.S.	530/387, 388; 435/7, 188, 240.27; 935/104,108,110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

Databases: SIN International Online (File CA, 1967-1990-File Biosis, 1969-1990) Automated Patent System (File USPAT, 1975-1990). For: monoclonal antibody, amyloid, cerebrovascular, protein/polypeptide, and Alzheimers

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X Y	US, A, 4,666,829 (GLENNER ET AL.) 19 May 1987 see entire document.	1-15 16-23
Y	Robakis et al., "Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neurity plaque amyloid peptides", Proc. Natl. Acad. Sci vol. 84: 4190-4194, Published June 1987. see especially p. 4192, Fig 2.	1-23
Y	Selkoe et al., "Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease" Science 235: 873-877, Published 20 February 1987. see especially page 874, column 1.	1-23
Y	Glenner et al., "Alzheimers disease:Initial report of the purification and characterization of a novel cerebrovascular amyloid protein" Biochem. Biophys. Res. Comm. 120: No.3, 885-890. Published 16 May 1984. see especially page 889.	1-23
Y	Glenner et al., "Alzheimer's disease and Down's Syndrome: sharing of a unique cerebrovascular amyloid fibril protein" Biochem. Biophys. Res. Comm. 122: 1131-1135. Published 16 August 1984. see especially pages 1131 and 1133, Table 1.	1-23

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 JUNE 1990	27 AUG 1990
International Searching Authority	Signature of Authorized Officer
ISA/US	PAULA HUTZELL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Kang et al., "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor" Nature, vol. 325: 733-736 Published 19 February 1989. see especially page 735.	1-23
Y	Wong et al., "Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related" Proc. Natl. Acad. Sci. USA 82: pages 8729-8732. Published December 1985. see especially page 8729.	1-23
Y	Goding, "Monoclonal antibodies: Principles and Practice" Published 1983 by Academic Press, Inc. (LONDON), see pages 56-97 and pages 209-249, especially pages 230-235.	1-23

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the International application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.